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Sbp1p Affects Translational Repression and Decapping in *Saccharomyces cerevisiae*

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The relationship between translation and mRNA turnover is critical to the regulation of gene expression. One major pathway for mRNA turnover occurs by deadenylation, which leads to decapping and subsequent 5′-to-3′ degradation of the body of the mRNA. Prior to mRNA decapping, a transcript exits translation and enters P bodies to become a potential decapping substrate. To understand the transition from translation to decapping, it is important to identify the factors involved in this process. In this work, we identify Sbp1p (formerly known as Ssb1p), an abundant RNA binding protein, as a high-copy-number suppressor of a conditional allele in the decapping enzyme. Sbp1p overexpression restores normal decay rates in decapping-defective strains and increases P-body size and number. In addition, Sbp1p promotes translational repression of mRNA during glucose deprivation. Moreover, P-body formation is reduced in strains lacking Sbp1p. Sbp1p acts in conjunction with Dhh1p, as it is required for translational repression and P-body formation in *pat1*Δ strains under these conditions. These results identify Sbp1p as a new protein that functions in the transition of mRNAs from translation to an mRNP complex destined for decapping.

Control of mRNA stability plays a crucial role in regulating gene expression. In eukaryotes, there are two major mRNA decay pathways that both require deadenylation before decay occurs. In yeast, the most common mechanism of mRNA turnover is where deadenylation is followed by decapping. Once the mRNA is deadenylated, the 5′ m7G cap structure is removed by the Dcp1p/Dcp2p decapping complex and the mRNA is subsequently degraded 5′ to 3′ (12, 20, 33). In addition to the decapping enzyme complex, there are a set of factors that act as decapping activators, including Dhh1p, Pat1p, and the Lsm1-7p complex (4, 10, 18, 44, 45). Alternatively, once deadenylation occurs, the mRNA can be degraded by the slower 3′-to-5′ pathway, which is mediated by the exosome (1, 5, 34; for a review, see reference 47).

Decapping is an important step in 5′-to-3′ decay, as it permits transcript degradation and is a site of regulation. For instance, short-lived mRNAs are decapped more rapidly than long-lived mRNAs (33, 34). In addition, nonsense transcripts, which are targeted for nonsense-mediated decay, can bypass the need for deadenylation and are rapidly decapped (35). Furthermore, in mammalian cells, the AU-rich element binding proteins TTP and BRF-1 have been proposed to recruit the decapping enzyme to their targets (31). Given these observations, to understand differential mRNA stability, it is important to determine the events that contribute to the control of mRNA decapping.

Several lines of evidence indicate that a key contribution to the control of mRNA decapping is that translation and mRNA decapping are in competition. First, in yeast, mRNAs poorly translated because of secondary structures in their 5′ untranslated region (UTR) or weak AUG context are decapped much faster than their wild-type counterparts (27, 34). Second, mutations in translation initiation factors such as eIF4E, the cap binding protein, and Prt1p, which is part of the eIF3 complex, also lead to faster degradation of mRNAs in yeast (39). Third, eIF4E inhibits the decapping activity of Dcp2p in vitro (38).

Evidence that there is a relationship between translation and mRNA decapping also comes from the analysis of P bodies (also referred to as Dcp1 bodies or GW182 bodies). P bodies are discrete cytoplasmic foci that contain both nontranslating mRNA and mRNA-decapping factors and are sites where decapping can occur (11, 41). P bodies are conserved in yeast and mammals in that both yeast and mammalian P bodies contain both subunits of the decapping enzyme and decapping activators (11, 14, 22, 30, 41, 46). However, mammalian P bodies differ from yeast P bodies in that they can contain translation initiation factors (14, 26). In addition, mammalian P bodies also contain the RNA binding protein GW182 and the Argonaute proteins, which are involved in micro-RNA function (14, 23, 28, 29). When mRNAs exit translation, they accumulate in P bodies to be targeted for degradation, as disruption of translation has an effect on the size and number of P bodies (43). For instance, trapping mRNAs in translation by using the elongation inhibitor cycloheximide results in loss of P bodies, while blocking of translation initiation results in an increase in both the size and number of P bodies, and decapping is accelerated (3, 39, 41, 43). Furthermore, two independent pathways have recently been identified for translational repression of mRNAs in yeast, and simultaneous disruption of these pathways leads to loss of P-body formation (9). Thus, P-body formation correlates with the status of translation in the cell. It is unclear whether the large P bodies visualized by fluorescence microscopy are required for decapping, or if decapping can occur in smaller P-body complexes containing the decapping machinery.

Recent work has begun to elucidate the factors and mech-

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TABLE 1. Strains used in this study

Strain	Genotype	Reference or source
yRP684	*MATα leu2-3,112 lys2-201 his4-359 trp1 ura3-52*	17a
yRP840	*MAT***a** *his4-539 leu2-3,112 trp1 ura3-52 cup1Δ::LEU2/PGK1pG/MFA2pG*	17a
yRP1068	*MAT***a** *his4-539 leu2-3,112 trp1 ura3-52 dcp1Δ::URA3 cup1::LEU2/PGK1pG/MFA2pG*	3a
yRP1345	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp1-2::TRP1 ski8Δ::URA3*	13a
yRP1346	*MAT***a** *his4-539 leu2-3,112 trp1 lys2-201 ura3-52 dcp2Δ::TRP1 cup1Δ::leu2/PGK1pG/MFA2pG*	3a
yRP1410	*MAT***a** *trp1 his4-539 ura3-52 leu2-3,112 lsm1Δ::TRP1 cup1Δ::LEU2/PGK1pG/MFA2pG*	44
yRP1502	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp2-7::URA3 ski3Δ::TRP1*	13a
yRP1515	*MATα his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp1-2::TRP1 cup1Δ::LEU2/PGKpG/MFA2pG*	13a
yRP1516	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp2-7::URA3 cup1Δ::LEU2/PGKpG/MFA2pG*	13a
yRP1529	*MATα ade2 his3 leu2-3,112 lys2 trp1 ura3-52 sbp1Δ::NEO cup1Δ::LEU2/PGKpG/MFA2pG*	This study
yRP1560	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dhh1Δ::URA3 cup1Δ::LEU2/PGKpG/MFA2pG*	10
yRP1600	*MATα his4-539 leu2-3,112 trp1 ura3-52 pat1Δ::LEU2 cup1Δ::LEU2/PGKpG/MFA2pG*	44
yRP1724	*MAT***a** *his4-539 leu2-3,112 trp1 ura3-52 cup1Δ::LEU2/PGK1pG/MFA2pG DHH1-GFP* (*NEO*)	42
yRP1727	*MAT***a** *his4-539 leu2-3,112 trp1 ura3-52 cup1Δ:LEU2/PGK1pG/MFA2pG DCP2-GFP* (*NEO*)	42
yRP2117	*MATα his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp12::TRP1 cup1Δ::LEU2/PGKpG/MFA2pG* [*SBP1* 2μm/*URA3*]	This study
yRP2118	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp2-7::URA3 cup1Δ::LEU2/PGKpG/MFA2pG* [*SBP1* 2μm/*TRP1*]	This study
yRP2119	*MAT***a** *his4-539 leu2-3,112 trp1 ura3-52 cup1Δ::LEU2/PGK1pG/MFA2pG* [*SBP1* 2μm/*URA3*]	This study
yRP2120	*MAT***a** *his4-539 leu2- 3,112 trp1 ura3-52 dcp1Δ::URA3 cup1Δ::LEU2/PGK1pG/MFA2pG* [*SBP1* 2μm/*TRP1*]	This study
yRP2121	*MAT***a** *trp1 his4-539 ura3-52 leu2-3,112 lsm1Δ::TRP1 cup1Δ::LEU2/PGK1pG/MFA2pG* [*SBP1* 2μm/*URA3*]	This study
yRP2122	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dhh1Δ::URA3 cup1Δ::LEU2/PGKpG/MFA2pG* [*SBP1* 2μm/*TRP1*]	This study
yRP2123	*MATα his4-539 leu2-3,112 trp1 ura3-52 pat1Δ::LEU2 cup1Δ::LEU2/PGKpG/MFA2pG* [*SBP1* 2μm/*URA3*]	This study
yRP2124	*MAT***a** *his4-539 lys2-201 leu2-3,112 trp1 ura3-52 dcp2Δ::TRP1 cup1Δ::LEU2/PGK1pG/MFA2pG* [*SBP1* 2μm/*URA*]	This study
yRP2125	*MAT***a** *his4-539 leu2-3,112 trp1 ura3-52 cup1Δ::LEU2/PGK1pG/MFA2pG DHH1-GFP* (*NEO*) [*SBP1* 2μm/*URA3*]	This study
yRP2126	*MAT***a** *his4-539 leu2-3,112 trp1 ura3-52 cup1Δ::LEU2/PGK1pG/MFA2pG DCP2-GFP* (*NEO*) [*SBP1* 2μm/*URA3*]	This study
yRP2127	*MAT***a** *leu2-3,112 his3/his4-539 trp1 ura3-52 lys2-201 sbp1Δ::NEO dhh1Δ::URA3cup1Δ::LEU2/PGK1pG/MFA2pG*	This study
yRP2128	*MAT***a** *leu2-3,112 his3/his4-539 trp1 ura3-52 sbp1 Δ::NEO lsm1Δ::TRP1 cup1 Δ::LEU2/PGK1pG/MFA2pG*	This study
yRP2129	*MATα leu2-3,112 his3/his4-539 trp1 ura3-52 ade2 sbp1Δ::NEO pat1Δ::LEU2 cup1::LEU2/PGK1pG/MFA2pG*	This study
yRP2130	*MATα leu2-3,112 lys2-201 his4-359 trp1 ura3-52* [*DCP2-GFP CEN/URA3*]	This study
yRP2133	*MATα his4-539 leu2-3,112 trp1 ura3-52 pat1Δ::LEU2 cup1Δ::LEU2/PGKpG/MFA2pG* [*DCP2-GFP CEN/URA3*]	This study
yRP2134	*MATα leu2-3,112 his3/his4-539 trp1 ura3-52 ade2 sbp1Δ::NEO pat1Δ::LEU2 cup1Δ::LEU2/PGK1pG/MFA2pG* [*DCP2-GFP CEN/URA3*]	This study
yRP2135	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp1-2::TRP1 ski8Δ::URA3* [*SBP1* 2μm/*LEU2*]	This study
yRP2136	*MAT***a** *his4-539 leu2-3,112 lys2-201 trp1 ura3-52 dcp2-7::URA3 ski8Δ* [*SBP1* 2μm/*LEU2*]	This study
yRP2137	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SBP1-GFP* (*NEO*)	This study

anisms by which mRNAs exit translation and assemble into a translationally repressed mRNP containing decapping factors. Several lines of evidence indicate that the decapping activators Dhh1p and Pat1p mediate the transition from translation to mRNA decay (9). First, overexpression of either Dhh1p or Pat1p causes global translational repression, as seen by decreased polysomes and a reciprocal increase in P bodies. Second, Dhh1p and Pat1p are activators of decapping, as loss of their activities leads to an increased mRNA half-life (10, 44). Third, strains lacking Dhh1p and Pat1p are defective for translational repression and subsequent P-body formation under conditions of glucose deprivation (9). It appears that both Dhh1p and Pat1p act independently for translational repression, as strains lacking either Dhh1p or Pat1p can still repress translation. This suggests that there are two potential mechanisms by which an mRNA can exit translation. An important goal is to further identify factors involved in this transition and the nature of their function.

In this work, we identify Sbp1p as a high-copy-number suppressor of both *dcp1-2* and *dcp2-7* mutations. Sbp1p, previously referred to as Ssb1p, was identified as a major single-stranded RNA binding protein containing two RNA recognition motifs that are separated by an RGG box (24, 25). It should be noted that the standard name for this factor in the yeast database is now Sbp1p, and we will refer to it by this name in this work. Sbp1p was initially localized to the nucleolus and can coimmunoprecipitate with snoRNA10 and snoRNA11, suggesting some function in ribosome biogenesis, although no specific defect in rRNA processing or ribosome biogenesis has been reported in *sbp1*Δ strains (8, 24, 25). We find that overexpression of Sbp1p can suppress mRNA decay defects in some decapping-defective strains. Furthermore, we

show that Sbp1p acts to promote translational repression of mRNA in conjunction with Dhh1p and subsequent mRNA localization to P bodies. We show here that Sbp1p plays a role in the transition of mRNA from translation to mRNA degradation.

MATERIALS AND METHODS

Strains and plasmids. All of the strains used in this study are listed in Table 1. The high-copy SBP1 2μm plasmids containing *URA3* (pRP1292) and containing *TRP1* (pRP1293) were created by PCR amplification of the *SBP1* gene from yRP840 containing 500 nucleotides upstream and 300 nucleotides downstream and ligated into vector pRS426 at the BglII and XhoI sites and into vector pRS424 at the SacI and XhoI sites, respectively. The *CEN*-containing pGAL-SBP1 (pRP1294) plasmid was created by PCR amplification of the *SBP1* gene from pRP1292 ligated into the XhoI and SalI sites of pRP23 downstream of the *GAL10* promoter. The *CEN*-containing *DCP2-RFP* plasmid (pRP1166) was constructed as described by Muhlrad and Parker (36). The *CEN*-containing *DCP2-GFP* plasmid containing *URA* (pRP1175) was constructed as described by Coller and Parker (9). The *CEN*-containing *DCP2-GFP* plasmid containing *TRP* (pRP1316) was constructed by restriction digestion of the EcoRI fragment containing *DCP2-GFP* from pRP1175 and ligation into the yCPlac22 vector.

The *Sbp1-GFP* fusion gene was obtained from Huh et al. (21) and contains the gene for the green fluorescent protein (GFP) fused to the 3′ end of the *SBP1* at its chromosomal location.

RNA isolation and analysis. All procedures with RNA were carried out as previously described by Muhlrad et al. (33). For half-life measurements, cells were grown to mid-log phase in medium containing 2% galactose. Cells were then harvested and resuspended in medium containing 4% glucose to repress transcription. Aliquots of cells were taken over a time course. RNA isolations were performed as described by Caponigro et al. (6). RNA was then analyzed by running on either a 1.25% formaldehyde agarose gel or on a 6% polyacrylamide–7.5 M urea gel. All Northern blots were then analyzed with a radiolabeled probe specific to MFA2pG (oRP140) or PGK1pG (oRP141) mRNA. Quantitation of half-lives was done with a Molecular Dynamics (Sunnyvale, CA) Phosphorimager. All samples were subsequently normalized for loading by hybridization and quantitation of 7S RNA (oRP100).

Microscopy. For microscopy, cells were grown to either mid-log phase (optical density [OD] = 0.3 to 0.4) or to a high OD (OD = 12.0). For cells grown to mid-log phase, cells were grown at 30°C, harvested, and washed and resuspended with minimal medium containing 2% glucose or minimal medium lacking glucose. Cells grown to a high OD were grown at 30°C in medium containing 2% glucose. They were subsequently harvested, washed, and resuspended in minimal medium with complete amino acids without glucose. Observations were done as described by Coller and Parker and Sheth and Parker (9, 41).

Polysome analysis. Polysome analyses were done as described by Coller and Parker (9). For galactose-induced overexpression of Sbp1p, cells were initially grown to mid-log phase in medium containing 2% sucrose. Cells were then harvested and resuspended in 2% galactose to induce Sbp1p overexpression or resuspended in 2% sucrose as a control for 2 h. Cells were harvested and frozen at −80°C. For glucose deprivation experiments, cells were grown to mid-log phase, harvested, and then resuspended in medium containing 2% glucose or in medium lacking glucose for 20 min. Cells were then harvested and frozen at −80°C. Extracts were then made and fractionated on 5 to 50% sucrose gradients as described by Coller and Parker (9).

[^{35}S]methionine incorporation assay. Cells were grown in 2% glucose to mid-log phase, harvested, and resuspended in medium either containing 2% glucose or lacking glucose for 20 min. [^{35}S]methionine was then added to each culture, and aliquots of cells were taken over a time course. [^{35}S]methionine incorporation assays were performed as described by Ashe et al. (2). The [^{35}S]methionine incorporation rate was measured by plotting values and measuring the slope in the linear range with a best-fit line.

RESULTS

Sbp1p is a high-copy-number suppressor of decapping defects. In order to identify other unknown modulators of decapping, we screened for high-copy-number suppressors of the temperature-sensitive *dcp2-7* allele. This screen is based on the fact that defects in the decapping enzyme are synthetically lethal with defects in 3′-to-5′ decay at 37°C, the nonpermissive temperature for *dcp2-7* (1). For instance, both *dcp2-7* and *ski3*Δ strains grow at 24°C and 37°C, whereas a *dcp2-7 ski3*Δ strain grows at 24°C but is dead at 37°C (13). We screened three genome equivalents of a yeast 2μm genomic library for high-copy-number suppressors of the lethal phenotype of the *dcp2-7 ski3*Δ strain at 37°C. Twenty-six transformants suppressed the lethal phenotype at 37°C. Of these, 24 contained a plasmid encoding DCP2 and two contained a plasmid encoding SBP1 (Table 2). However, growth was not restored in a vector-only control (yEP351) (Table 2).

TABLE 2. Growth analysis of yeast strains used in this study[a]

Strain	Temp (°C)	Growth phenotype
Wild type	37	+++
*dcp2-7 ski3*Δ	37	−
*dcp2-7 ski3*Δ *SBP1* 2μm	37	++
*dcp2-7 ski3*Δ *yEP351*	37	−
Wild type	33	+++
*dcp1-2 ski8*Δ	33	−
*dcp1-2 ski8*Δ *SBP1* 2μm	33	++
*dcp1-2 ski8*Δ *yEP351*	33	−
Wild type	35	+++
SBP1 2μm	35	+++
*pat1*Δ	35	−
*pat1*Δ *SBP1* 2μm	35	++
*dhh1*Δ	35	−
*dhh1*Δ *SBP1* 2μm	35	−
*lsm*Δ	35	−
*lsm1*Δ *SBP1* 2μm	35	−

[a] The growth of various strains at various temperatures was analyzed in plating assays. −, no growth; + through +++, weak growth to strong growth at the restrictive temperatures shown.

To determine if Sbp1p overexpression can suppress defects in the decapping enzyme complex, we determined whether Sbp1p overexpression could suppress a conditional allele in the other decapping enzyme subunit, the *dcp1-2* allele. *dcp1-2*, like *dcp2-7*, is also synthetically lethal, with defects in 3′-to-5′ decay at its nonpermissive temperature. To this end, we overexpressed Sbp1p by transforming the *dcp1-2 ski8*Δ strain with a high-copy-number, 2μm plasmid encoding SBP1. We then looked for suppression of the lethal phenotype of the *dcp1-2 ski8*Δ strain at 33°C, the nonpermissive temperature for the *dcp1-2* allele (13). Overexpression of Sbp1p allowed the *dcp1-2 ski8*Δ strain to grow at the nonpermissive temperature, whereas the *dcp1-2 ski8*Δ strain carrying the empty vector (yEP351) did not grow (Table 2). This indicates that Sbp1p overexpression suppresses the synthetic lethality associated with defects in the decapping enzyme complex in combination with defects in 3′-to-5′ decay.

Sbp1p overexpression suppresses decapping defects in *dcp1-2* and *dcp2-7*. To determine how Sbp1p overexpression suppresses the lethal phenotype of the double mutants, we examined whether Sbp1p overexpression suppresses defects in mRNA turnover. Sbp1p overexpression could suppress the lethal phenotype by either enhancing the 5′-to-3′ mRNA decay pathway or 3′-to-5′ mRNA decay. In the first assay, we analyzed the unstable MFA2pG reporter, which contains a

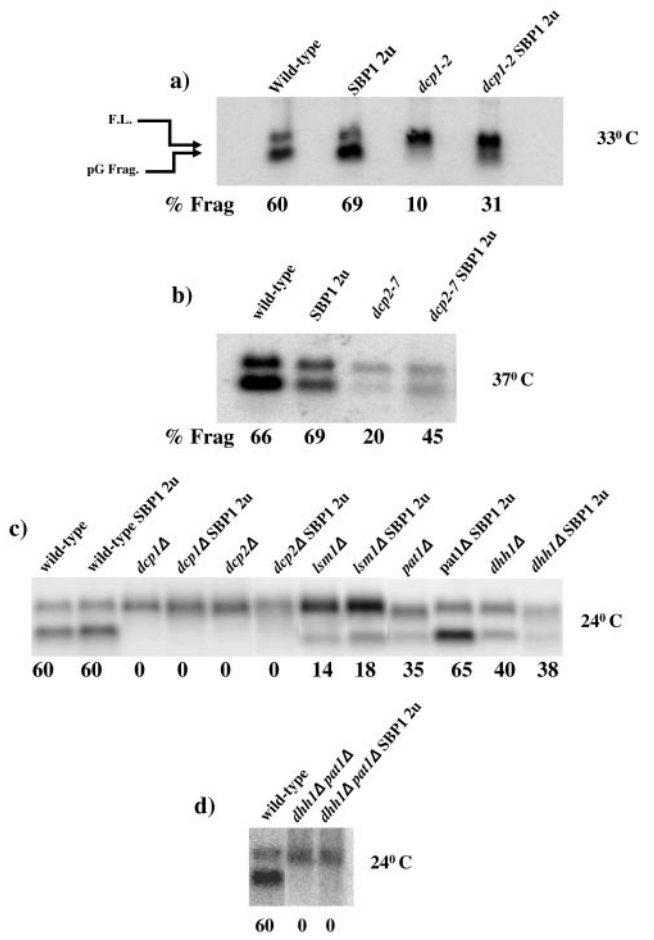

FIG. 1. Sbp1p overexpression suppresses the full-length (F.L.) to pG fragment (Frag.) defect. The ratio of full-length MFA2pG reporter to the pG fragment is shown and is a simple measurement of decapping activity. (a) Steady-state distributions in strains containing *dcp1-2* at the nonpermissive temperature of 33°C. (b) Steady-state distributions in strains containing *dcp2-7* at the nonpermissive temperature of 37°C. (c) mRNA distributions for strains with mutations in the decapping enzyme (*dcp1*Δ and *dcp2*Δ) or strains with mutations in the decapping activators (*dhh1*Δ, *lsm1*Δ, and *pat1*Δ). (d) Steady-state distributions for *dhh1*Δ *pat1*Δ and *dhh1*Δ *pat1*Δ SBP1 2μm strains. Zero denotes less than 5% fragment. The temperatures at which the experiments were performed are noted next to the panels.

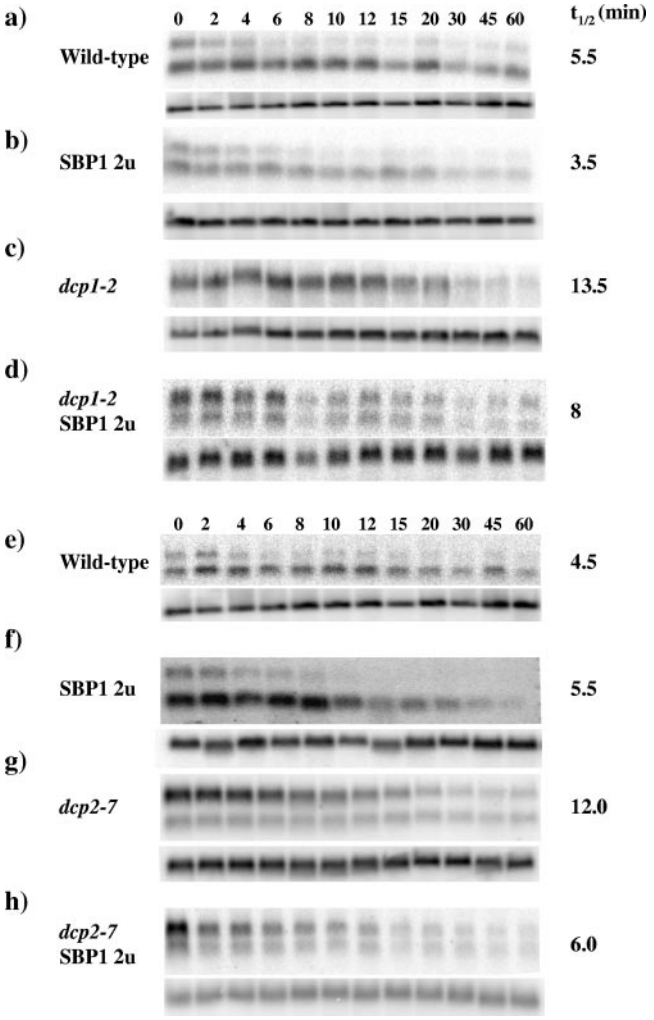

FIG. 2. Sbp1p overexpression suppresses mRNA half-life ($t_{1/2}$) defects in conditional decapping mutants. Agarose Northern blot assays were done for the MFA2pG reporter mRNAs for the wild-type (a), SBP1 2μm (b), *dcp1-2* (c), and *dcp1-2* SBP1 2μm (d) strains at 33°C and for the wild-type (e), SBP1 2μm (f), *dcp2-7* (g), and *dcp2-7* SBP1 2μm (h) strains at 37°C. Time points after transcriptional repression are indicated above the lanes. The top band in each panel is full-length MFA2pG mRNA, and the bottom band is the pG fragment. The 7S RNA shown below each panel was used as a loading control. Half-lives were determined with the full-length MFA2pG mRNA band and are indicated beside the panels. All experiments were done a minimum of three times.

poly(G) tract within the MFA2 3′ UTR (12). This poly(G) tract blocks 5′-to-3′ exonucleolytic decay and thereby traps decay intermediates. Therefore, the ratio of mRNA decay intermediate (pG fragment) to the full-length transcript is a simple measurement of the effectiveness of the 5′-to-3′ decay pathway (5, 12).

For this experiment, we again used the conditional *dcp1-2* and *dcp2-7* alleles in decapping. Cells were grown to mid-log phase at 30°C and shifted to the nonpermissive temperatures (33°C for experiments with the *dcp1-2* allele and 37°C for experiments with the *dcp2-7* allele) for 1 h. In a wild-type strain, we saw that the ratio of full-length transcript to pG fragment was approximately 40% to 60% whereas in the decapping-defective *dcp1-2* and *dcp2-7* strains, we found that the ratios of full-length transcript to pG fragment were 90% to 10% and 80% to 20%, respectively (Fig. 1a and b). When we overexpressed Sbp1p in the *dcp1-2* and *dcp2-7* strains, we found that fragment levels increased threefold and twofold, respectively (Fig. 1a and b). This suppression was dependent upon the decapping complex, as Sbp1p overexpression did not suppress the defect in the *dcp1*Δ and *dcp2*Δ strains (Fig. 1c). This result is consistent with Sbp1p overexpression increasing 5′-to-3′ decay by facilitating the activity of the partially defective decapping enzymes in the *dcp1-2* and *dcp2-7* strains at the nonpermissive temperatures.

Sbp1p overexpression suppresses half-life defects of decapping-defective strains. In a second assay, we tested whether Sbp1p overexpression restores normal mRNA decay rates in

the *dcp1-2* and *dcp2-7* mutant strains (Fig. 2). Again, we utilized the MFA2pG transcript, which is under the control of the inducible galactose promoter, and grew cells to mid-log phase in galactose. For the *dcp1-2* strain, we shifted to the nonpermissive temperature of 33°C for 1 h and then repressed transcription by adding glucose. In a *dcp1-2* strain, we observed that the MFA2pG mRNA has a half-life of approximately 14 min at 33°C, compared to that in the wild-type strain, where the half-life is approximately 5 min at 33°C. More importantly, Sbp1p overexpression suppressed the half-life defect in a *dcp1-2* strain at the nonpermissive temperature such that the half-life of MFA2pG is approximately 8 min (Fig. 2a to d). Similar results were obtained with a *dcp2-7* strain. For strains containing the *dcp2-7* allele, we grew cells to mid-log phase in galactose, shifted to the nonpermissive temperature of 37°C for 1 h, and then repressed transcription by adding glucose. In a *dcp2-7* strain, we observed an MFA2pG mRNA half-life of approximately 12 min, and upon Sbp1p overexpression, the MFA2pG half-life is approximately 6 min (Fig. 2e to h). We note that the decay rates for the MFA2pG mRNA are different for the wild-type strain overexpressing Sbp1p (Fig. 2b and f). This difference in decay rate is most likely due to the different nonpermissive temperatures used for the *dcp1-2* allele (33°C) and the *dcp2-7* allele (37°C). Nonetheless, Sbp1p overexpression partially restores decay rates in conditional decapping mutants.

There are two possibilities by which Sbp1p could accelerate mRNA turnover rates in conditional decapping-defective strains. Sbp1p overexpression could facilitate the decapping step, or Sbp1p overexpression could increase the deadenylation rate in *dcp1-2* and *dcp2-7* strains. To distinguish between these possibilities, we measured the rates of poly(A) shortening in polyacrylamide Northern blot assays. Sbp1p overexpression had no effect on the deadenylation rates of MFA2pG mRNA in either a *dcp1-2* or a *dcp2-7* strain at the nonpermissive temperatures (data not shown). In combination, the above results are consistent with Sbp1p overexpression suppressing decapping defects in the conditional mutants.

Sbp1p overexpression suppresses mRNA-decapping defects in a *pat1*Δ strain but not a *dhh1*Δ or an *lsm1*Δ strain. To gain insight into how Sbp1p overexpression affects decapping, we examined whether Sbp1p overexpression suppresses the decapping defects seen in strains lacking the decapping activator Lsm1p, Dhh1p, or Pat1p. Again, we examined the ratio of full-length MFA2pG mRNA to the pG decay fragment. Upon Sbp1p overexpression, we found that the defect in pG fragment accumulation was suppressed in a *pat1*Δ strain but not in a *dhh1*Δ or an *lsm1*Δ strain (Fig. 1c). Consistent with these results, Sbp1p overexpression was able to suppress the temperature-sensitive growth phenotype in a *pat1*Δ strain but not in a *dhh1*Δ or an *lsm1*Δ strain (Table 2). Thus, Sbp1p overexpression suppresses the decapping defects in a *pat1*Δ strain but not in a *dhh1*Δ or an *lsm1*Δ strain.

Interestingly, recent results show that there are two independent manners by which mRNAs move from translation and are targeted for decapping, one dependent on Dhh1p and the other one dependent on Pat1p (9). Since Sbp1p overexpression suppresses the pG fragment accumulation defect in a *pat1*Δ strain, it suggests that Sbp1p may act either in conjunction with Dhh1p or downstream of Pat1p to promote decapping.

To test these two possibilities, we determined whether Sbp1p overexpression could suppress the decapping defect in a *dhh1*Δ *pat1*Δ strain. If Sbp1p were acting in conjunction with Dhh1p, then Sbp1p overexpression would not suppress the defect in accumulation of the pG fragment. On the contrary, if Sbp1p were downstream of Pat1p, then Sbp1p overexpression would suppress the pG fragment accumulation phenotype in this strain. In a *dhh1*Δ *pat1*Δ strain, there was virtually no accumulation of the pG fragment, and upon Sbp1p overexpression, we saw no suppression of this defect (Fig. 1d). This result suggests that Sbp1p acts in conjunction with Dhh1p to promote decapping.

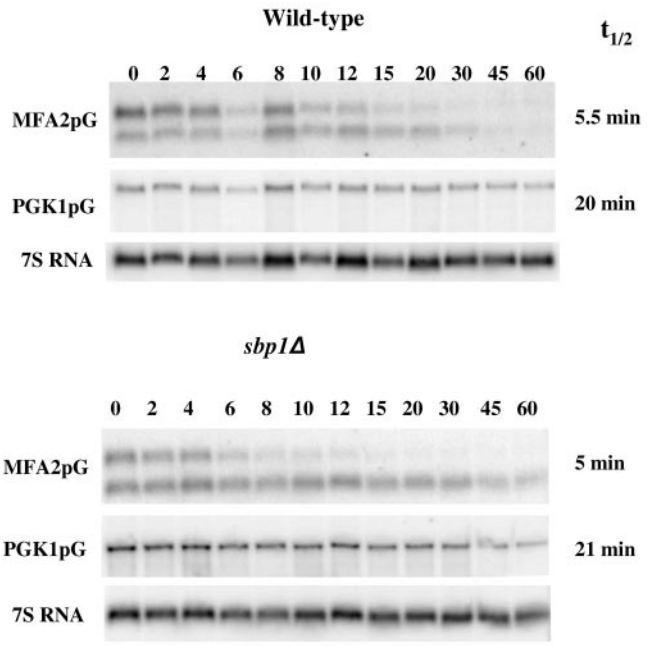

FIG. 3. Sbp1p is not required for normal rates of mRNA decay. Both MFA2pG and PGK1pG mRNA decay rates in both wild-type and *sbp1*Δ mutant strains are shown. Time points after transcriptional repression are shown above the lanes. The 7S RNA shown was used as a loading control. Half-lives ($t_{1/2}$) are indicated beside the panels. All experiments were done a minimum of three times.

Sbp1p is not required for normal rates of decapping. The above results indicate that Sbp1p overexpression affects the rate of mRNA decapping in strains where decapping is partially compromised. To determine if Sbp1p is required for mRNA decapping, we examined the decay rate of the MFA2pG and PGK1pG mRNAs in an *sbp1*Δ strain. We observed that an *sbp1*Δ strain shows MFA2pG and PGK1pG mRNA decay rates similar to those of a wild-type strain (Fig. 3). This result suggests that Sbp1p is not required for mRNA decapping.

However, Sbp1p may be involved in a non-rate-limiting step in mRNA decapping. Thus, the function of Sbp1p may be revealed when *sbp1*Δ is combined with other mRNA-decapping defects. Given this possibility, we examined the effects of *sbp1*Δ in *dcp1-2* and *dcp2-7* strains at 24°C, where *dcp1-2* and *dcp2-7* strains are slightly compromised for mRNA decapping. We observed that *sbp1*Δ has no effect on the MFA2pG or PGK1pG mRNA decay rate even when combined with the *dcp1-2* or *dcp2-7* allele (data not shown). In addition, we ob-

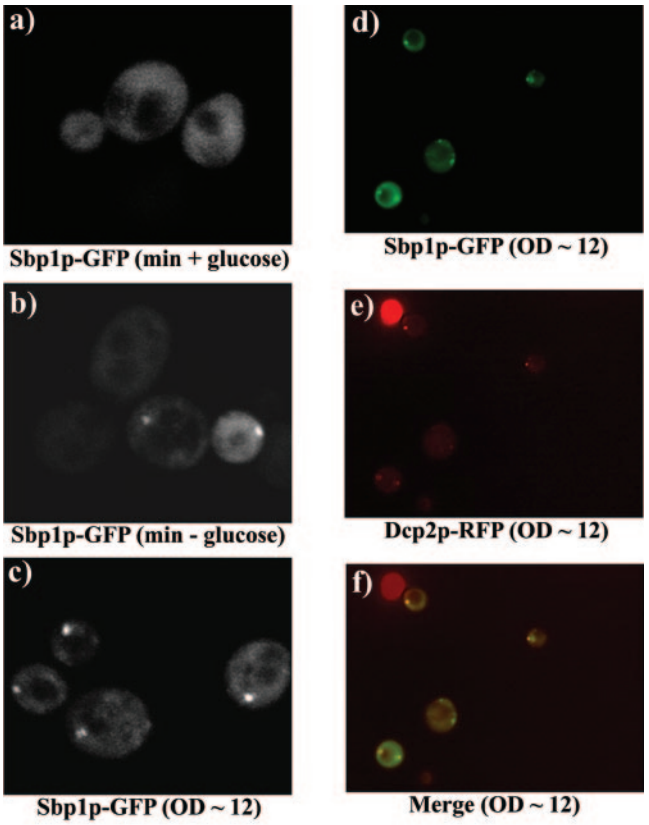

FIG. 4. Sbp1p localizes to P bodies under stress conditions. SBP1-GFP cells were grown to log phase and resuspended in minimal (min) medium containing dextrose (a), grown to log phase and resuspended in minimal (min) medium lacking dextrose (b), or grown to a high OD (OD, ~12) (c). (d to f) SBP1-GFP cells containing the DCP2-RFP plasmid were grown to a high OD (OD ~12). Panels: d, Sbp1p-GFP; e, Dcp2p-RFP; f, merge.

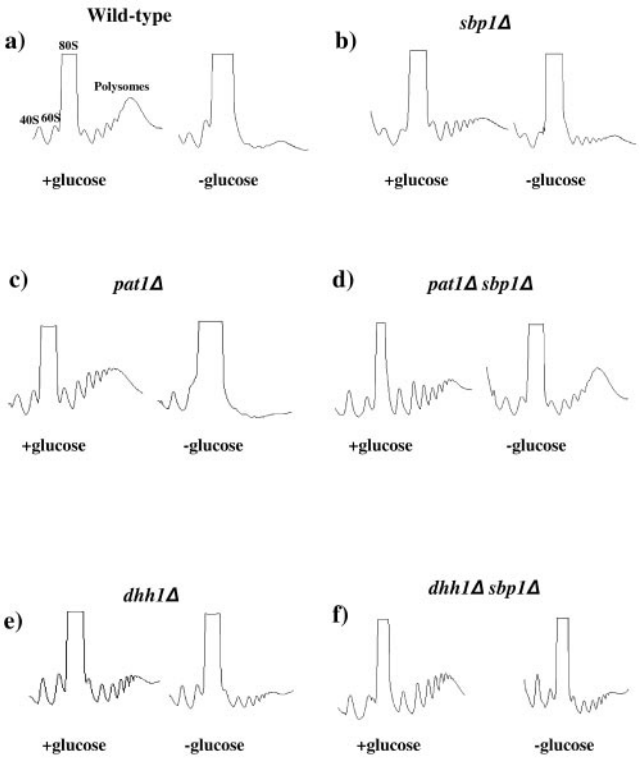

FIG. 5. *sbp1*Δ fails to repress translation after glucose deprivation when put in combination with *pat1*Δ but not *dhh1*Δ. Shown are polysome profiles (OD, A_{260}) of the yeast strains grown continuously in glucose (+glucose) and after glucose deprivation (−glucose). Panels: a, wild type; b, *sbp1*Δ; c, *pat1*Δ; d, *pat1*Δ *sbp1*Δ; e, *dhh1*Δ; f, *dhh1*Δ *sbp1*Δ. All experiments were done a minimum of three times.

served that *sbp1*Δ also has no effect on the MFA2pG or PGK1pG decay rate when combined with *lsm1*Δ, *pat1*Δ, or *dhh1*Δ (data not shown). These results suggest that Sbp1p does not have a global effect on mRNA decapping. Although the loss of Sbp1p does not appear to have any effect on decay rates per se, this raises the possibility that Sbp1p normally enhances decapping on only a subset of messages but, when overexpressed, has a more general effect on mRNA decapping.

Sbp1p localizes to P bodies under stress conditions. Proteins that affect the decapping process, such as Dcp1p, Dcp2p, Dhh1p, Pat1p, and Lsm1p, localize to P bodies, which are sites in the cell where decapping may occur (11, 14, 22, 30, 41, 43, 46). As Sbp1p overexpression affects the decapping process, we asked whether Sbp1p-GFP localizes to P bodies under several growth conditions (Fig. 4). For this experiment, we used a strain with a wild-type genetic background, in which GFP is fused to the C terminus of the endogenous SBP1 and is driven off its own promoter (21). In cells at mid-log phase, Sbp1p-GFP appears evenly distributed throughout the cytoplasm (100% of cells; $n = 77$) (Fig. 4a). At mid-log phase, P bodies are generally small and not all proteins that localize to P bodies are easily visualized in them. However, under stress conditions, P bodies become large and proteins that localize to P bodies are easily visualized in them. For that reason, we grew cells to mid-log phase and then shifted them to medium lacking glucose or we looked at high cell density. Under glucose deprivation stress, we saw Sbp1p-GFP in one to two foci per cell in 21% of the cells examined ($n = 56$ cells) (Fig. 4b). At high cell density, we saw Sbp1p-GFP in one to two foci per cell in 77% of the cells examined ($n = 110$ cells) (Fig. 4c). Furthermore, we colocalized Sbp1p-GFP with Dcp2p-RFP at high cell density (82% of cells; $n = 46$), confirming that Sbp1p is a component of P bodies (Fig. 4d to f). The fact that Sbp1p can accumulate in P bodies is consistent with Sbp1p affecting the decapping process.

Sbp1p promotes translational repression in conjunction with Dhh1p. One mechanism by which Sbp1p overexpression can act to increase decapping is by enhancing the decapping step directly. On the other hand, another possibility is that Sbp1p overexpression promotes translational repression, which then leads to increased decapping. Previous work has identified two factors that act in translational repression, Pat1p and Dhh1p, which promote translational repression independently (9). Sbp1p overexpression suppresses decapping defects in *pat1*Δ strains but not in *dhh1*Δ and *dhh1*Δ *pat1*Δ strains, suggesting that Sbp1p might act in conjunction with Dhh1p for translational repression. Therefore, we asked whether Sbp1p promotes translational repression either alone or in conjunction with Dhh1p or Pat1p.

To ask whether Sbp1p promotes translational repression, we

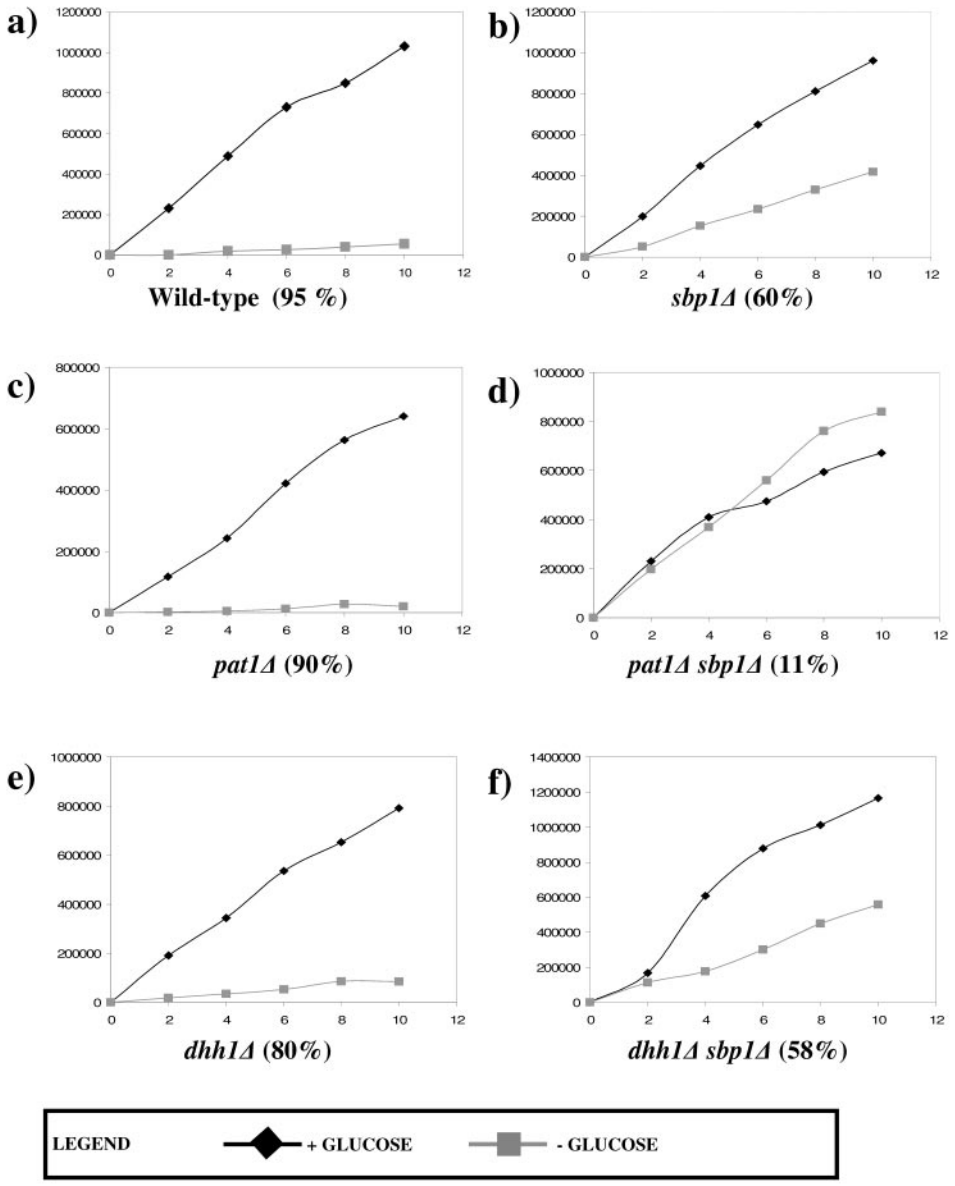

FIG. 6. Translation rate is affected in *sbp1*Δ strains after glucose deprivation. Incorporation of [^{35}S]methionine in various yeast strains grown continuously in glucose (diamonds) and after glucose deprivation (squares) in wild-type (a), *sbp1*Δ (b), *pat1*Δ (c), *pat1*Δ *sbp1*Δ (d), *dhh1*Δ (e), and *dhh1*Δ *sbp1*Δ (f) strains. Time is marked on the *x* axis in minutes, and incorporation is marked on the *y* axis in counts per minute. The percent decrease in the [^{35}S]methionine incorporation rate of cells resuspended in medium lacking glucose compared to that of cells grown continuously in glucose is noted next to each genotype. All experiments were done a minimum of three times.

determined whether loss of SBP1 function would result in inhibition of translational repression. One simple manner of examining translational repression is to study cells under glucose deprivation, where translation declines and mRNAs concentrate in P bodies (2, 19, 43). To this end, we examined translation by both polysome profiles and studying [^{35}S]methionine incorporation rates in both wild-type and *sbp1*Δ strains under conditions of glucose deprivation (Fig. 5a and b and 6a and b). For both assays, we grew cells to mid-log phase and then shifted cells to medium lacking glucose for 20 min. We then assayed polysome profiles and [^{35}S]methionine incorporation rates. In wild-type strains, we observed that translation was almost completely repressed by glucose deprivation, as polysomes markedly decreased and the [^{35}S]methionine incorporation rate was reduced by 95% compared to that found under glucose-rich conditions (Fig. 5a and 6a). Interestingly, in an *sbp1*Δ strain, polysomes decreased slightly less than in a wild-type strain (Fig. 5a and b). Furthermore, the [^{35}S]methionine incorporation rate was reduced by only 60% compared to that found under glucose-rich conditions (Fig. 6b). The increased polysomes and [^{35}S]methionine incorporation rate seen in the *sbp1*Δ strain, compared to a wild-type strain, suggest that loss of Sbp1p can at least partially impair translational repression under glucose-lacking conditions.

To determine whether Sbp1p acts in conjunction with Dhh1p for translational repression, we looked at polysomes and [^{35}S]methionine incorporation rates in both *pat1*Δ *sbp1*Δ and *dhh1*Δ *sbp1*Δ double mutants under glucose deprivation (9; this work). If Sbp1p acts in conjunction with Dhh1p, then in a *pat1*Δ *sbp1*Δ strain we would expect to see additive defects, as both translational repression pathways would be blocked. By contrast, in a *dhh1*Δ *sbp1*Δ strain, we would expect the phenotype to be similar to that of either the single-mutant *dhh1*Δ strain or the single-mutant *sbp1*Δ strain, as Pat1p would still be functional for translational repression (9).

Strikingly, a *pat1*Δ *sbp1*Δ strain was no longer able to repress translation as polysomes and [^{35}S]methionine incorporation rates were largely unaffected under glucose deprivation (Fig. 5d and 6d). The results obtained with a *pat1*Δ *sbp1*Δ strain were compared to those obtained with a *pat1*Δ strain, which still repressed translation under these same conditions, as seen by polysome analysis and by which the [^{35}S]methionine incorporation rate was reduced by 90% (Fig. 5c and 6c). Conversely, in a *dhh1*Δ *sbp1*Δ strain, both polysomes and the [^{35}S]methionine incorporation rate decreased under glucose deprivation (Fig. 5e and f and 6e and f). However, the *dhh1*Δ *sbp1*Δ strain showed a phenotype similar to that of an *sbp1*Δ strain, as the [^{35}S]methionine incorporation rate was reduced by 58%, whereas in a *dhh1*Δ strain the [^{35}S]methionine incorporation rate was reduced by 80% compared to that seen under glucose-rich conditions (Fig. 6b, e, and f). The fact that a *pat1*Δ *sbp1*Δ strain is defective for translational repression whereas a *dhh1*Δ *sbp1*Δ strain is still partially functional suggests that Sbp1p works in conjunction with Dhh1p.

***sbp1*Δ strains are defective in P-body formation during stress response.** As Sbp1p plays a role in translational repression in conjunction with Dhh1p, we asked if *sbp1*Δ affects the formation of P bodies either by itself or in combination with *dhh1*Δ or *pat1*Δ. If translational repression were inhibited, P bodies would show a reduced number and size as mRNA is retained in the translating pool. Since Sbp1p plays a role in translational repression under stress, we grew *sbp1*Δ cells to a high OD (a glucose-limiting condition) and ascertained what happens to P bodies by visualization with Dcp2p-GFP (43). For this experiment, *dcp2-GFP* is contained on a *CEN* plasmid and expressed off its own promoter (9). These experiments yielded several interesting observations. First, wild-type cells show one to three large P bodies per cell (80% of cells, $n = 84$). Interestingly, *sbp1*Δ cells show one to four small P bodies per cell (88% of cells, $n = 60$), which are significantly reduced in size compared to wild-type cells (Fig. 7a and b). This result is consistent with Sbp1p having an effect on translational repression on its own (Fig. 6b).

Similarly, in *pat1*Δ cells, there are one to two small P bodies per cell (85% of cells, $n = 90$) (Fig. 7c). Thus, the P bodies in *pat1*Δ cells are reduced in size and number compared to those in wild-type cells but are still present (Fig. 7a and c). When we looked at *pat1*Δ *sbp1*Δ cells, we found that there is zero to one P body per cell (83% of cells, $n = 75$), which is further reduced in size compared to *pat1*Δ cells (Fig. 7c and d). In contrast, when we visualized P bodies in *dhh1*Δ cells, we found three to five P bodies per cell (89% of cells, $n = 105$) that appeared only slightly smaller than those in wild-type cells (Fig. 7a and e). In *dhh1*Δ *sbp1*Δ cells, we found that there is a reduction in the number of P bodies per cell compared to *dhh1*Δ cells, such that there are two to three P bodies per cell (89% of cells, $n = 110$), which are similar in size to those in *dhh1*Δ cells (Fig. 7e and f). Therefore, both *dhh1*Δ cells and *dhh1*Δ *sbp1*Δ cells show roughly similar accumulations of P bodies, while we see a reduction in both the size and number of P bodies in *pat1*Δ *sbp1*Δ cells compared to those in *pat1*Δ cells. The fact that P bodies are reduced more in an *sbp1*Δ strain than in a *dhh1*Δ *sbp1*Δ strain is likely explained by the decapping defect seen in *dhh1*Δ strains, which could lead to a slightly greater accumulation of mRNAs in P bodies. Nevertheless, these results are consistent with Sbp1p working in conjunction with Dhh1p to mediate translational repression.

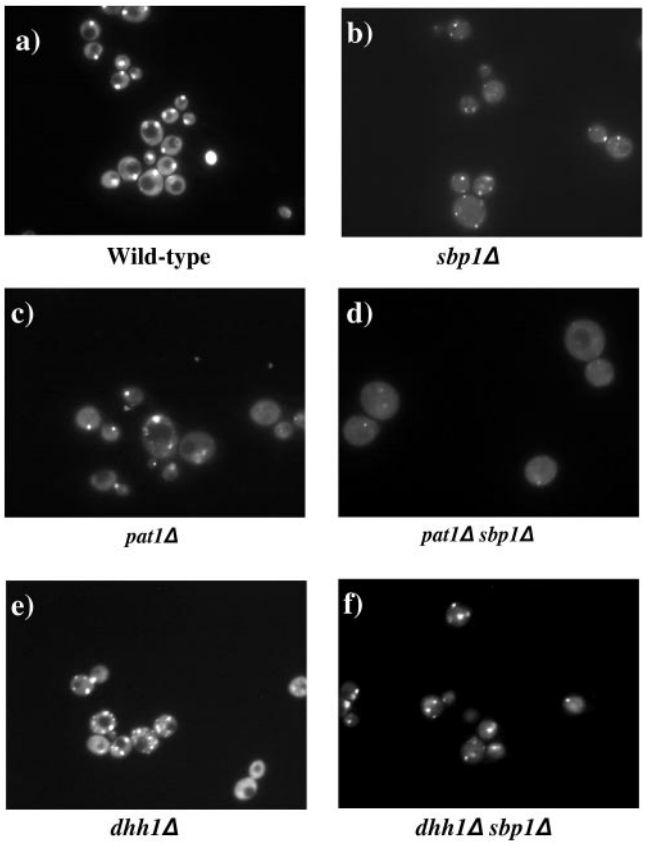

FIG. 7. Sbp1p and Pat1p have additive effects on P-body formation. With Dcp2p-GFP as a marker for P bodies, we observed P-body formation at a high OD in wild-type (a), *sbp1*Δ (b), *pat1*Δ (c), *pat1*Δ *sbp1*Δ (d), *dhh1*Δ (e), and *dhh1*Δ *sbp1*Δ (f) strains.

Sbp1p overexpression promotes translational repression and increases the number and size of P bodies. Since loss of Sbp1p results in increased translation and a reduction in P bodies both on its own and in combination with loss of Pat1p under glucose deprivation, we expected Sbp1p overexpression to reduce polysomes and promote P-body formation. Therefore, we overexpressed Sbp1p by placing it under control of the galactose-inducible promoter. We grew cells to mid-log phase in medium containing sucrose and then shifted cells to medium containing galactose for 2 h. We then assayed for translational repression by polysome analysis (Fig. 8). Upon galactose induction, we saw that polysomes were markedly reduced in a wild-type strain overex-

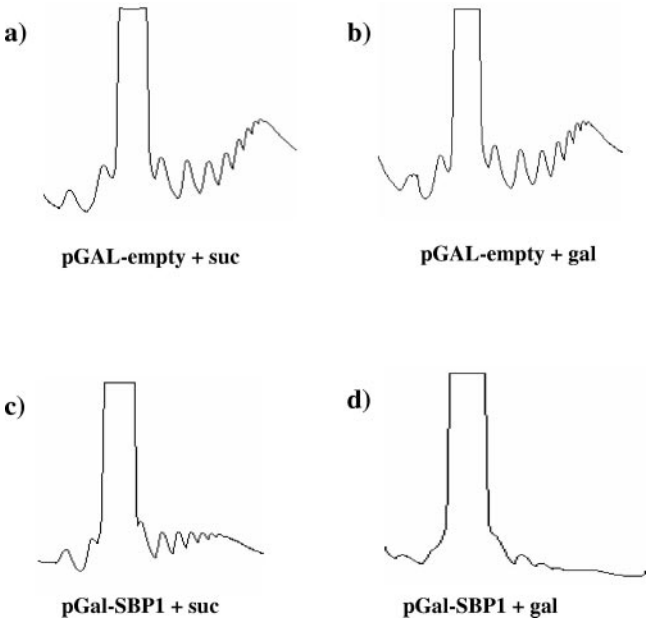

FIG. 8. Overexpression of Sbp1p causes translational repression. Shown are polysome profiles from control cells carrying an empty vector (pGAL-empty) and cells carrying an Sbp1p overexpression vector (pGAL-SBP1). pGAL-empty cells grown in sucrose (suc) (a), pGAL-empty cells grown in galactose (gal) (b), pGAL-SBP1 cells grown in sucrose (c), and pGAL-SBP1 cells grown in galactose to induce Sbp1p overexpression (d) were tested. All experiments were done a minimum of three times.

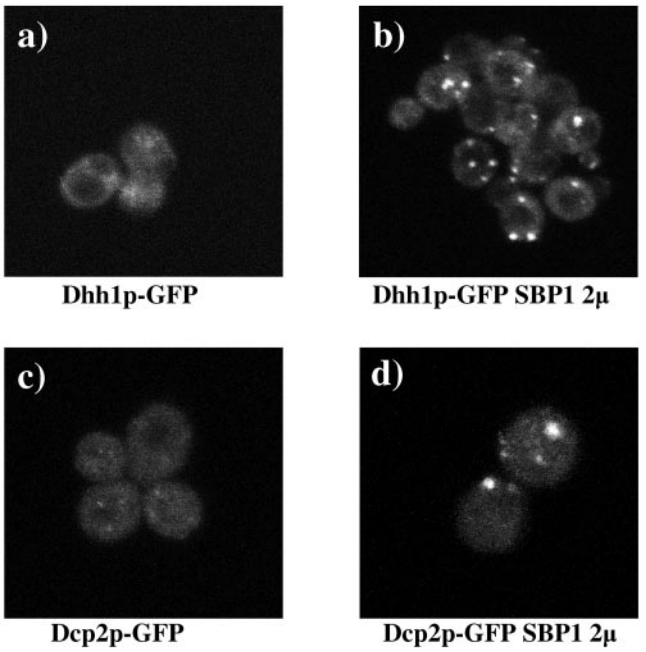

FIG. 9. Sbp1p overexpression affects the number and size of P bodies. SBP1 was overexpressed in strains carrying endogenous GFP-tagged markers for P bodies. Panels: a, DHH1-GFP; b, DHH1-GFP SBP1 2μm; c, DCP2-GFP; d, DCP2-GFP 2μm.

pressing Sbp1p (pGAL-SBP1), compared to a wild-type strain carrying an empty vector (pGAL-empty) (Fig. 8b and d). Moreover, the decrease in polysomes in the pGAL-SBP1 strain was due to induction of Sbp1p overexpression as both pGAL-empty and pGAL-SBP1 still contained polysomes when uninduced (Fig. 8a and c). These results are consistent with Sbp1p promoting translational repression.

Since Sbp1p overexpression promotes translational repression, we asked whether Sbp1p overexpression increases P-body size and number. This would be analogous to how Dhh1p and Pat1p function, as overexpression of either results in a decrease in polysomes and an increase in P bodies (9). To examine the effects of Sbp1p overexpression on P-body size and number, we looked at the subcellular localization of P-body markers Dhh1p-GFP and Dcp2p-GFP (41, 43). Both strains were created by fusing GFP to the C terminus of the endogenous gene (41). In cells grown to mid-log phase and resuspended in medium containing glucose, Dhh1p-GFP is distributed throughout the cell and is rarely seen in P bodies (90% of cells, $n = 40$) (43). However, when we overexpressed Sbp1p, we found a dramatic increase in the presence of Dhh1p in P bodies (80% of cells, $n = 78$) (Fig. 9a and b). In addition, at mid-log phase, P bodies appear small when visualized by Dcp2p-GFP (73% of cells, $n = 56$), and when we overexpressed Sbp1p, we also observed an increase in Dcp2p-GFP localization in P bodies (79% of cells, $n = 67$) (Fig. 9c and d). Taken together, these results show that the enhanced decapping in strains overexpressing Sbp1p is caused by translational repression and an increase in mRNA concentrated in P bodies.

DISCUSSION

Sbp1p globally promotes translational repression of mRNAs. In this work, we initially identified Sbp1p as a high-copy suppressor of decapping mutants. However, several observations suggest that Sbp1p affects the transition of mRNAs from the translating pool to the nontranslating pool, where they accumulate in P bodies. First, translational repression is attenuated in *sbp1*Δ strains under glucose deprivation, as both polysomes and [^{35}S]methionine incorporation rates decrease in *sbp1*Δ strains, but less than in the wild type (Fig. 5). Moreover, the defect in translational repression is increased in *pat1*Δ *sbp1*Δ strains under glucose deprivation, as polysomes fail to decrease and the [^{35}S]methionine incorporation rate is largely unaffected. In contrast, polysomes and [^{35}S]methionine incorporation rates are only moderately affected in *dhh1*Δ *sbp1*Δ strains under glucose deprivation, suggesting that Sbp1p promotes translational repression as part of the Dhh1p pathway. Additionally, P bodies are reduced under stress conditions in *sbp1*Δ strains and are almost nonexistent in *pat1*Δ *sbp1*Δ strains, which correlates with Sbp1p modulating the transition of mRNAs from translation as part of the Dhh1p pathway (Fig. 5, 6, and 7). Furthermore, Sbp1p overexpression decreases polysomes, which correlates with a reciprocal increase in P bodies (Fig. 8 and 9). Lastly, Sbp1p localizes to P bodies under conditions of limiting glucose or at a high cell density, which is consistent with promoting translational repression of mRNAs and targeting them to P bodies (Fig. 4). Taken together, our results indicate that Sbp1p promotes translational repression of mRNAs and their subsequent localization to P bodies, where they can become decapping substrates.

Sbp1p overexpression promotes decapping. As Sbp1p affects the transition of mRNA from translation to a P body, which is a discrete cytoplasmic focus where decapping factors localize, Sbp1p may also affect mRNA decapping (11, 41). Previous results show that both Dhh1p and Pat1p promote translational repression of mRNA and that these factors also play a role in subsequent mRNA decapping (9, 10, 44). Thus, we have several lines of evidence that indicate that Sbp1p may play a role in mRNA decapping. First, Sbp1p overexpression suppresses the growth defects when conditional mutations for decapping are combined with defects in 3′-to-5′ mRNA decay (Table 2). For instance, Sbp1p overexpression suppresses the synthetic lethal phenotype of *dcp2-7 ski3*Δ and *dcp1-2 ski8*Δ strains at the nonpermissive temperatures (Table 2). Second, Sbp1p overexpression suppresses both the pG fragment accumulation defects and half-life defects at the restrictive temperatures for both *dcp2-7* and *dcp1-2* mutants. However, it should be noted that an *sbp1*Δ strain shows no defects in mRNA decay rates of the MFA2pG and PGK1pG reporters under normal growth conditions (Fig. 3). This suggests that Sbp1p is not required for the decapping step and that only when overexpressed does Sbp1p affect decapping. It is possible, however, that Sbp1p may promote decapping for only a subset of messages.

Sbp1p shows similarity to mammalian nucleolin. Sbp1p does not have any definitive homologues but shows similarity to mammalian nucleolin, a shuttling protein involved in both ribosome biogenesis and regulation of mRNA translation and stability (7, 25, 40, 42; for a review, see reference 17). Indeed, mammalian nucleolin acts specifically in regulating the stability and translation of the interleukin-2 mRNA through its 5′ UTR, repressing p53 translation through its 5′ UTR, and the stability of the bcl-2 mRNA through AU-rich elements in its 3′ UTR (7, 40, 42). Sbp1p shows homology with nucleolin in both the RNA binding domains and the RGG (glycine-arginine) box, which are involved in both RNA binding and protein-protein interactions (8, 24, 25). The possibility that Sbp1p shows similarity to nucleolin leaves the idea that nucleolin may also be found in P bodies in mammalian cells.

Interestingly, previous work by Clark et al. and Jong et al. found that Sbp1p (initially termed Ssb1p) was localized to the nucleolus and was proposed to be involved in ribosome biogenesis (8, 25). Furthermore, only overexpression of Sbp1p resulted in its localization to the cytoplasm (8). In contrast, our work shows Sbp1p-GFP evenly distributed throughout the cell at mid-log phase (Fig. 4), which is consistent with Sbp1p-GFP having a cytoplasmic localization, as seen in the genomic studies (21). We also observed that at a high cell density or during glucose deprivation, Sbp1p-GFP accumulates in P bodies (Fig. 4). However, we do not observe a concentrated nucleolar localization pattern above the levels of the cytoplasmic signal (Fig. 4). The difference in these results could be due to the use of fixed versus live cells or the detection reagents utilized (8). We also note that the antibody used in the initial studies cross-reacts with two other species, and previous immunofluorescence studies do not show the nucleolar staining seen with the Sbp1p antisera to be lacking in an *sbp1*Δ strain (8, 24, 25). This raises the possibility that the previously seen concentration of Sbp1p in the nucleolus might have been due to the cross-reacting species, perhaps because Sbp1p shares an RGG box, which is characteristic of some nucleolar proteins. However, as Sbp1p-GFP is localized throughout the cell, our results do not necessarily conflict with the previously proposed role of Sbp1p acting in the nucleolus in ribosome biogenesis. This is similar to nucleolin, which can act in translational repression in the cytoplasm and have a function in the nucleolus (7, 17, 42).

Potential models of Sbp1p action. There are two general models by which Sbp1p may work to promote translational repression. Given that Sbp1p is an RNA binding protein, it is possible that Sbp1p directly binds mRNA and inhibits the function of translation initiation factors. Proteomic studies show that Sbp1p copurifies with both eIF4E and eIF4G (15, 16). eIF4G and eIF4E are members of the cytoplasmic cap binding complex and are responsible for recruiting the 40S ribosomal subunit to the mRNA for scanning (for reviews, see references 32 and 37). Perhaps the interaction of Sbp1p with these factors leads to destabilization of eIF4E and eIF4G from the cap, which would then lead to translational repression. A second possibility is that Sbp1p directly binds the mRNA and facilitates the full assembly of the translational repression complex. This would subsequently lead to association of other factors involved in translational repression and delivery of the mRNA to a P body. One implication of this model is that an intermediate mRNP complex may form between translation and a P body which contains Sbp1p. Sbp1p would then dissociate from this mRNP before localization to a P body. Consistent with this idea, Sbp1p is the first factor identified where loss of its activity impairs translational repression but does not have a generalized effect on mRNA decapping. Additionally, Sbp1p does not localize to P bodies under log-phase growth conditions (10, 44). Only under stress conditions does Sbp1p localize to P bodies, and this may be due to the fact that the rate of flux of mRNAs into P bodies may be changed. Both models suggest that Sbp1p may act early in the transition from translation to a P body, as it can interact with translational initiation factors, and it localizes to P bodies in a manner similar to that of Dhh1p, a factor which acts early in this transition, perhaps by inhibiting the activity of the 40S ribosome (9, 15, 16). It should be noted that the models presented here are not mutually exclusive and that a reasonable hypothesis is that Sbp1p acts to destabilize translation initiation complexes and stays associated with the mRNA, facilitating assembly of the translational repression complex.

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